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Discovery and Characterization of Potent and Selective AXL Receptor Tyrosine Kinase Inhibitor AB801

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OVERVIEW

- AXL receptor tyrosine kinase (AXL) is a transmembrane protein that is overexpressed in a variety of tumors and levels of which correlate with poor prognosis in cancer patients. AXL is expressed by cancer and stromal cells and has been implicated in the development of resistance to chemotherapy, targeted therapies, and immunotherapies.
- Arcus Biosciences has discovered and characterized AB801: a novel, highly potent and selective small-molecule AXL inhibitor.
- This poster describes the discovery and characterization of AB801, designed and optimized with the aid of structure-based drug design. AB801 was characterized in multiple *in vitro* assays and *in vivo* studies.

AXL BIOLOGY & DOWNSTREAM EFFECTS

- AXL expression is correlated to proliferation, migration/invasion, and epithelial-mesenchymal transition (EMT) in cancer cells. Overexpression can cause drug resistance during cancer treatments and attenuate immune responses.[1,2]
- Activation and dimerization of AXL by its ligand, growth arrest specific protein 6 (GAS6), or ligand-independent dimerization facilitates AXL phosphorylation.
- AXL can homodimerize or heterodimerize independently of GAS6, also leading to phosphorylation.
- AXL phosphorylation initiates signaling cascades that promote cancer cell proliferation, survival, and an immunosuppressive microenvironment.

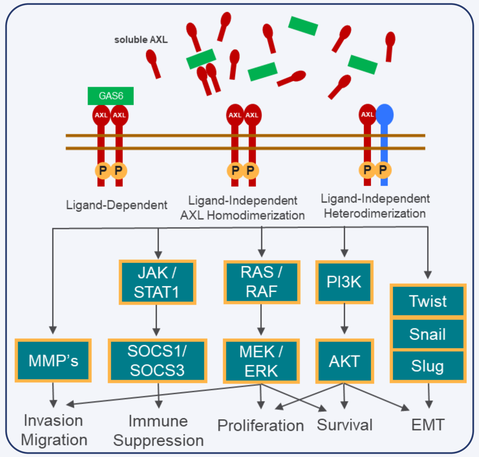

Figure 1. Binding of GAS6 to AXL facilitates dimerization, subsequent phosphorylation, and signaling through various pathways that promote cancer cell survival. Ligand-independent dimerization can also occur.

INITIAL DESIGN, OPTIMIZATION, AND CHARACTERIZATION OF AB801

Structural Biology and Rationale for AXL Receptor Tyrosine Kinase Inhibition

AXL receptor signaling can be blocked using three main strategies: inhibition of dimerization through use of an antibody (Figure 2A), reduction of dimerization utilizing a GAS6 trap (Figure 2B), or blockade of receptor auto-phosphorylation with small molecule inhibitors of the intracellular kinase domain (Figures 2C).[3]

We embarked upon the design of a novel AXL inhibitor first through examination of existing academic and commercial small molecules, followed by structure-based drug design, and finally SAR optimization of multiple assay readouts. Our primary goals were to discover an inhibitor which has excellent potency within a novel structural space, an excellent safety profile, and good pan-kinase selectivity. We specifically sought to avoid MERTK and TYRO3 inhibition to reduce any potential off-target effects.[4] Due to the high homology of these catalytic domains, design of AXL-selective inhibitors is challenging.[5]

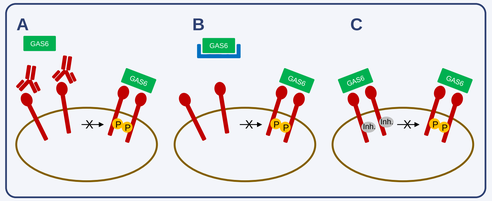

Figure 2. Selected strategies for inhibiting AXL phosphorylation. a) Blocking antibody to prevent dimerization. b) GAS6 trap (blue) to limit dimerization. c) Small molecule inhibition of kinase domain to prevent auto-phosphorylation.

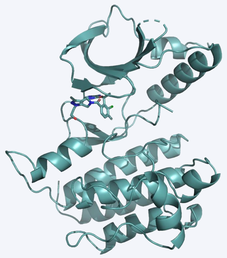

Figure 3. X-ray structure of AXL/small molecule inhibitor complex (PDB: 5U6B).[5]

Characterization and Comparison of AB801 and Benchmark AXL Inhibitors

	Assay	AB801	Bemcentinib[6] (BGB324, R428)	Dubermatinib[6] (DS1205b, AB-329)	Sitravatinib[6]
biochemical	hAXL HTRF IC_{50} (700 µM ATP)	3.3 nM	5.2 nM	77 nM	4.0 nM
	mAXL / rAXL / dAXL HTRF IC_{50} (700 µM ATP)	0.43 / 0.29 / 0.38 nM	7.2 / 6.4 / 14 nM	--	--
	hAXL K_i	0.024 nM	0.25 nM	--	--
	Fold selectivity over hMERTK / hTYRO3 (enzyme K_i over AXL K_i)	860x / 1400x	130x / 400x	14x / 9x[7]	4x / 0.3x[7]
cellular	pAXL ELISA IC_{50} (serum-free media)	17 nM	270 nM	--	--
	pAXL ELISA IC_{50} (100% serum)	68 nM	1,100 nM	--	--

Table 1. Characterization and comparison of optimized Arcus and benchmark AXL inhibitors. Kinase activity of AXL was determined using a HTRF KinEASE – TK kit (CisBio) in the presence of 700 µM ATP. K_i values against AXL, MERTK, and TYRO3 were determined using Cheng-Prusoff analysis. Dose-dependent inhibition of AXL activation was determined using pAXL ELISA in HEK293T cells transiently transfected with Nanoluc-tagged AXL. After 1 hr compound incubation, cells were lysed and auto-phosphorylated AXL was captured by anti-phosphoryl tyrosine antibody and detected by NanoLuc activity. Compound incubation was carried out in either serum-free or 100% human serum media.

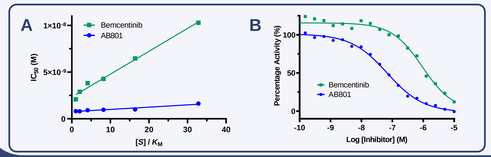

Figure 4. A) Determination of AXL K_i values for AB801 and Bemcentinib using Cheng-Prusoff analysis. **B)** Inhibition of AXL by AB801 and Bemcentinib in pAXL ELISA cellular assay with 100% serum.

Pan-Kinase Selectivity of AB801

In addition to being selective against MERTK and TYRO3, AB801 was tested against a broad non-mutant kinase panel to determine any other potential off-target liabilities.[8] Using a qPCR-based competition binding experiment, AB801 showed <35% of control activity (indicating stronger binding) against 7 other kinase targets, excluding MERTK and TYRO3. Follow-up K_d values were determined through qPCR-based competition binding dose response.

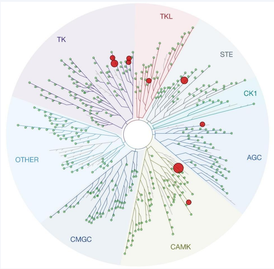

Figure 5. Kinome map (403 total) showing significant kinase/AB801 interactions in red.

Kinase	K_d	$K_{d,Kin}/K_{d,AXL}$
AXL	0.093 nM	1
DRAK1	4.4 nM	47
HPK1	26 nM	280
TRKA	31 nM	330
RPS6KA4	130 nM	1,400
ACVR1	170 nM	1,800
TRKB	300 nM	3,000
MAST1	>1000 nM	>10,000

Table 2. K_d values for AB801 with kinases showing <35% of control activity.

Preclinical Pharmacokinetic Characterization

AB801 was found to have low to moderate clearance and an acceptable half-life in all preclinical species tested. AB801 has high oral bioavailability in rats and exposure is dose-proportional.

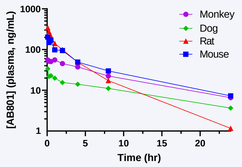

Figure 6. Mean plasma concentration after IV dosing of AB801 in preclinical species.

Parameter	Species			
	Mouse	Rat	Dog	Monkey
CL (L/h/kg)	0.54	0.65	1.5	0.76
Vss (L/kg)	4.4	2.4	28	10
$T_{1/2}$ (h)	7.6	4.0	13	11
MRT (h)	8.2	3.7	18	13

Table 3. Pharmacokinetic parameters of AB801 in preclinical species.

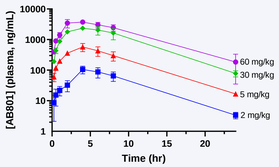

Figure 7. Mean plasma concentration after oral dosing of AB801 in rats at increasing concentrations.

Dose (mg/kg)	C_{max} (ng/mL)	AUC_{inf} (h·ng/mL)
2	110	1,100
5	570	5,700
30	2,400	28,000
60	4,200	45,000

Table 4. Pharmacokinetic parameters with oral dosing of AB801 in rats.

PROJECTED HUMAN PK AND SAFETY

Projected Human Pharmacokinetics

AB801 exhibits a favorable *in vitro* pharmacokinetic profile with low intrinsic clearance in human hepatocytes (Table 5). AB801 is predicted to exhibit moderate clearance, long half-life, and good oral bioavailability in humans (Table 6).

Hepatocyte Stability

	Mouse	Rat	Dog	Monkey	Human
CL_{int} (µL/min/10^6 cells)	2.8	23	2.5	2.7	1.8

Table 5. Summary of hepatocyte stability in preclinical species.

Projected Human PK Parameters

CL (L/h/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	Bioavailability (%)
0.46[a]	16[b]	24	60

Table 6. Projected human pharmacokinetic profile of AB801. [a]Well-stirred model with adjustment for underprediction based on preclinical animal data (IVIVC/IVIVE). [b]Øie-Tozer method.

Safety of AB801

- AB801 displays a good safety profile, with limited direct and time-dependent CYP inhibition; additional assays show limited CYP induction. Taken together, there is a low potential for DDI.
- hERG inhibition at 1 and 10 µM was 8% and 48%, respectively, supportive of a large therapeutic window.

SUMMARY

- Through structure-based drug design and SAR optimization, AB801 was identified as a small molecule AXL inhibitor.
- As characterized in functional biochemical and cell-based assays, AB801 exhibits excellent potency and selectivity. Compared to previous small-molecule AXL inhibitors, AB801 shows an improved profile.
- AB801 shows an excellent pan-kinase selectivity profile, supportive of a very low probability for any off-target liabilities. The lowest K_d ratio is for DRAK1 (47-fold), with all other kinases being >100-fold.
- Good pharmacokinetic properties are observed in preclinical species with dose-proportional exposure seen in rats.
- AB801 is predicted to exhibit moderate clearance, long half-life, and good oral bioavailability in humans. A good safety profile is noted, with low DDI or hERG liabilities.

REFERENCES AND NOTES

1) Zhu, *et al. Mol. Cancer* **2019**, 18:153.
2) Son, *et al. Front. Oncol.* **2021**, 11:756225.
3) Sang, *et al. Front. Oncol.* **2022**, 12:811247.
4) Hamm, *et al. Arch. Toxicol.* **2022**, 96:613.
5) Gajiwala, *et al. J. Biol. Chem.* **2017**, 292(38):15705.
6) Data generated by Arcus. Compounds purchased from Synnovator (Bemcentinib), Advanced ChemBlocks (Dubermatinib), and Cayman Chemical (Sitravatinib).
7) MERTK or TYRO3 HTRF IC_{50} divided by AXL HTRF IC_{50} (700 µM ATP).
8) Analysis performed at Eurofins DiscoverX Corporation (San Diego, CA, USA).